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ASSESSING THE LOSS OF MICROVASCULAR ARCHITECTURE AND INTRANEURAL FIBROSIS: VITAL FINDING IN FAILED HUMAN NERVE ALLOGRAFTS

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ABSTRACT

Background: Processed nerve allografts are commonly used for clinical nerve repair. Nonetheless, chronic pain and ongoing loss of function have been linked to allograft failure, which has a high documented prevalence rate. Goal: One year after the initial operation, the current study sought to evaluate the unsuccessful allograft reconstruction in a sensory human nerve by immunohistochemical and histological investigation.

Methods: Reconstruction using processed nerve allografts was performed on four patients who had suffered superficial radial nerve injuries. Clinical findings at the follow-up visit revealed significant neuropathic pain and no reinnervations of sensory nerves. The removal of the unsuccessful transplant and histologic and immunohistochemical analyses came next. Collagen content, lymphatic and blood vasculature, and the neurofilament network were measured in the middle of the specimens.

Results: Histologic examination revealed increased fibrosis, fatty degeneration, and disordered growth of nerve fibers. Additionally, a recognizable pattern was observed in the microvascular network of the allografts, with an increase in microvessels and no alteration in the lymphatic vasculature.

Conclusion: Within the constraints of the research, the current study finds that loss of microvascular and physiologic architecture is linked to human allograft failure. More clinical research is necessary to evaluate the interaction between angiogenesis, lymphangiogenesis, and axonal regeneration, nevertheless, in order to better understand the mechanism underlying the failure of human nerve allografts.

Keywords: peripheral nerve, nerve surgery, lymphatic drainage, and nerve allograft.

INTRODUCTION

Damage to peripheral nerves is a frequent and highly prevalent condition that affects a sizable portion of the global human population, including those in India. Severe nerve injuries, however, come with a heavy cost, both socially and financially, since the long-term effects cause the affected individuals to stop working and take time off.1. Despite numerous advances in microsurgery techniques, neuropathic pain remains a significant barrier in reconstructive nerve surgical, along with the loss of motor and sensory skills. Nerve continuity can be restored using synthetic/autologous conduits or allogenic/autologous nerve grafts in situations where tension-free neurorrhaphy is not

practical. The paradigm in peripheral nerve reconstruction changed as a result of the growing use of processed nerve allografts, even if autografts were still recognized as the gold standard of care. Nevertheless, harvesting the autograft has a number of negative effects, including as scarring, neuroma development, loss of feeling, and morbidity at the donor site. Conversely, using allografts has none of these issues or disadvantages. Furthermore, processed nerve allografts do not require further immunosuppression because they do not include antigenicity from the original cell components. Neural microarchitecture is preserved in the allografts, providing guidance and a scaffold for the regeneration of axons between severed nerve terminals.3.

Despite the encouraging outcomes and significant recovery observed in earlier research utilizing these allografts, graft failure due to neuroma formation and graft resorption in continuity can cause chronic discomfort, hypesthesia, and persistent paresthesia.4 The exact biological mechanisms behind transplant failure are unknown, although inadequate blood flow to the allograft—which can result in scarring and graft necrosis—is thought to be a critical issue. According to evidence from the literature now in publication, intraneural angiogenesis promotes Schwann cells in terms of cellular alignment and produces neurotrophic factors, both of which are essential for axonal regeneration.
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According to findings from the recent literature, peripheral nerve regeneration should be associated with both angiogenesis and lymphangiogenesis, which denotes the restoration of neural lymphatic drainage. By providing more information about the mechanism that may restrict the processed nerve allografts' ability to successfully regenerate their nerves, the results of surgical care can be enhanced.Six The current clinical study was carried out in light of these published findings in order to evaluate the failure of allograft reconstruction using immunohistochemical and histological examination in a sensory human nerve one year after the initial surgery.

MATERIALS AND METHODS

One year after the initial operation, the current clinical investigation evaluated the unsuccessful allograft reconstruction in a sensory human nerve using immunohistochemical and histological examination. After receiving approval from the relevant ethical committee, the current study was conducted at... from.. to. Prior to beginning the study, informed consent was obtained from each study participant, both verbally and in writing. Four patients who had surgical treatment using processed nerve allografts were evaluated for the study. The radial artery, the extensor pollicis brevis and abductor pollicis longus tendons, and the superficial radial nerve were all impacted related to the trauma in both cases.

The current clinical inquiry used immunohistochemical and histological analysis to assess the unsuccessful allograft reconstruction in a sensory human nerve one year after the initial procedure. The present investigation was carried out at... from.. to after being given clearance by the appropriate ethical committee. Written and verbal informed consent was obtained from every study participant prior to the start of the investigation. The study analyzed four patients who had surgical therapy using processed nerve allografts. The trauma in both cases affected the superficial radial nerve, the radial artery, and the tendons of the extensor pollicis brevis and abductor pollicis longus.

.. In order to accomplish the procedure, surgical microscopes were used to examine severed stumps of the superficial radial nerve and evaluate the intraneural morphology. After the excision, an allograft was used to restore the nerve without strain, and fibrin glue and sutures were used to approximate the area. Following the five days of operation, wrist immobilization was indicated, and mobilization was carried out in accordance with hand therapy guidelines. Multidisciplinary care involving the pain specialists was initiated during the postoperative phase. All research participants received systemic or local anti-neuropathic pain drugs for three to six months following surgery, depending on the need.

Following a 12-week period, each subject was permitted to load to capacity. After a year of follow-up, the subjects were brought back, and the decision to proceed with a surgical reconstruction was based on the results of the radiographic evaluation and clinical examination, which indicated a growing neuropathic pain and advancing Tinel's sign. Perineural lipofilling was performed in all individuals to boost the revascularization of the nerve reconstructions and to obtain mechanical protection after a sample of SRN was collected from a healthy proximal nerve stump.

The healthy proximal superficial radial nerves were sectioned into slices about 2µm thick, and the allografts were fixed in formalin.

To be examined under a microscope, paraffin-embedded slides were stained with eosin and hematoxylin stains. To improve the collagen's visibility, an extra Elastin van Gieson stain is created. Monoclonal antibodies were utilized for immunochemical staining utilizing an automated immunostainer. Normal nerve fibers, blood arteries, and lymphatic vessels were considered the positive internal controls. Therefore, there was no need for external positive control.

RESULTS

After the initial nerve reconstruction, the study participants experienced significant and early allodynia with no sensory recovery in the superficial radial nerve region. Excision of the allografts following a 12-month follow-up period. The allografts were distinguished morphologically by thickening at the coaptation site and central atrophy. Two participants reported significant neuropathic pain at the 12-month follow-up after the resurgery, posing a risk of morbidity and incapacity, whereas the other two went back to work.

In terms of neurofilament and collagen distribution, histologic evaluation of removed allografts revealed higher levels of pathological neurofilaments and intraneural fibrosis compared to normal samples taken from the superficial radial nerves.

Along with the disorderly development of the neurofilaments, deteriorated allografts in two participants showed signs of fibrosis and lipomatosis. The predominant finding in the other two patients was intraneural fibrosis. When comparing immunohistochemistry results for neurofilaments to normal superficial radial nerve tissues, the results showed fewer and poorly ordered neurofilaments.

The superficial radial nerve's blood and lymphatic vasculature had distinctive hierarchical microvessels, primarily in the perineurium and epineurium. Conversely, the removed allograft displayed a greater quantity of randomly distributed micro-vessels. D2-40 was applied to the sections in order to evaluate the intraneural lymphatic network. Lymphatic channels were observed in the perineural and epineural soft tissues of normal superficial radial nerves. Lipomomatosis and fibrosis were observed in deteriorated allografts in two cases in addition to the

Also, in failed allografts, no increase in lymphangiogenesis was seen with scarce lymphatic vessels in perineural tissue.

DISCUSSION

Clinical outcomes for nerve injury reconstruction from the upper and lower limbs have been consistent. However, as recently described by Safa et al.7 in 2020 and Nietsovaara et al.8 in 2019, failure of these grafts can result in irreversible loss of function and accompanying chronic pain. Histologic evaluation of the explanted human allografts revealed central necrosis and inadequate axonal regrowth, which is in line with the results of the current investigation and the 2019 study by Leckenby et al9.

It is important to keep in mind, though, that the majority of the data regarding the failure of nerve allografts still comes from case studies and rodent experiments, and that the topic is obscured by the lack of a standard protocol for performing the histologic examination of failed nerve allografts. In order to recreate a sensory human nerve, four cases of unsuccessful nerve allografts were evaluated in the current clinical study. In addition, the study evaluated the collagen content, neurofilament network, and lymphatic and blood vasculature in the center of the examined specimens. As noted by Cattin et al.10 in 2015, the functional integration of the nerve grafts is a complicated event requiring revascularization and axonal ingrowth.

At the site of coaptation, significant scarring or intraneural fibrosis may impede the growth of axons and endothelial cells. Staining revealed core intraneural fibrosis in the current investigation, along with a disordered neurofilament architecture that was similar to a neuroma. These findings were consistent with a 2013 case report by Berrocal et al.11, which described a failed nerve transplant with around 6% regeneration at the core and axonal degradation from 16000 to 1000 fibers. This was comparable to a 2019 study by Nietsvaara et al.8 that showed how decellularized allograft and host rejection were likely contributing reasons to graft failure and how allograft failure led to graft resorption.

Recent research on revascularization of allografts was conducted by Saffari et al.12 in 2020, and they found insufficient axonal regrowth along with central graft resorption or necrosis. The revascularization of nerve allografts and autografts is caused by longitudinal inosculation, as shown by Zhu et al.'s 2017 illustration, which shows the ingrowing vessels to the remaining microvascular channels in the nerve end. Because the surviving vascular network in decellularized allografts lacks endothelial and mural cells, the technique is less practical. In addition, Zhu et al. (2013) found that allografts showed slower vascularization than autografts. The center of the failed allograft in the current study revealed a thick, disordered microvascular network that was not longitudinal.

Even though the unsuccessful allografts were removed after a year, the current study's findings may aid in understanding the process of central graft necrosis.

The process of nerve regeneration was described by Duboyy et al.(2011) as a complicated biologic entity involving a variety of cell lines, where VEGF produced by macrophages causes the polarized microvasculature, which aids in the growth and migration of Schwann cells' axons. Similar to this, the microvascular system plays a major role in stimulating neural regeneration by aiding in cell metabolism, providing trophic and nutritional factors, and aiding in different stages of the healing process by drawing in stem cells from the bloodstream, as demonstrated by Caillud et al.15 in 2019.

In addition to the well-established role of angiogenesis, Frueh et al.16 in 2020 suggested that lymphangiogenesis and the lymphatic system can play a critical role in peripheral nerve injury and nerve regeneration. Meng et al.'s 2020 confirmation of the lymphatic system's function in peripheral nerve repair comes lately. The lymphatic vasculature of normal superficial radial nerve and failed nerve allografts was assessed using a D2-40 stain. It was observed that in failed allografts and normal nerve, there was no alteration observed in comparison to blood vasculature.

Since the Resurgery was carried out after a year, it is challenging to evaluate the validity of these results, which calls for additional clinical longitudinal research to determine the lymphatic system's contribution to peripheral nerve regeneration. As proven by Frueh et al.16 in 2020, tissue edema may be considerably reduced with the activation of lymphangiogenesis in nerve allografts by the clearance of myelin debris, inflammatory cells, and interstitial fluid. This can lead to a better functional recovery and encourage the synthesis of new myelin.

A couple of the study's weaknesses were that it was descriptive in nature and that it used short, thin allografts, which could have led to different outcomes when using long, thick allografts. Additionally, the small sample size and brief follow-up may provide inconsistent findings.

CONCLUSION

Considering its limitations, the present study concludes that an abnormal neurofilament arrangement, fatty degeneration, and increased intraneural fibrosis are associated with failed nerve allografts. Also, the allografts have a disorganized and dense microvascular network with very few perineural and epineural lymphatic vessels. Further clinical studies are needed and vital for a better understanding of the interplay of axonal regeneration, lymphangiogenesis, and angiogenesis to decrease allograft failure risk in the future.

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